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A review on delignification of lignocellulosic biomass for enhancement of ethanol production potential

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ABSTRACT

In the recent past, significant research has been made by thermal, mechanical, chemical and microbial pretreatments in the process of delignification. Production of ethanol from the lignocellulosic material has been done in three major steps: (i) delignification; (ii) depolymerization and (iii) fermentation. Pretreatment has been one of the most expensive processing steps in cellulosic biomass to fermentable sugar conversion. Present review article presents recent advances in the field of delignification. Research article also comprehensively discusses the different pretreatment methods along with effect of delignification on ethanol production and the uses of lignin in different industries. It has been found out that; pretreatment methods have significant impact on production efficiency of ethanol from biomass. This further signifies that, the pretreatment results must be balanced against their impact on cost of the processing steps and the trade-off between operating costs, capital costs and biomass cost.

Contents

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1. Introduction

Conversion of abundant lignocellulosic biomass to ethanol as a transportation fuel presents an important opportunity to improve energy security, reduce the trade deficit, reduce greenhouse gas emission and improve price stability [1]. In last few decades several ways of utilizing biomass and associated waste for energy production in different forms e.g., biogas, bio-diesel, pyrolytic bio-oil, etc. have been envisaged thoroughly by researchers worldwide [2–6]. It becomes imperative to explain in beginning why ligno-cellulosic biomass should be converted to ethanol in comparison to other biofuels such as bio-diesel, pyrolytic oil, bio-gas and many others. This can be explained simply because of these main reasons delignification of lignocellulosic biomass becomes so lucrative and important for ethanol production because of its abundance and low cost. Also low oil content in lignocellulosic biomass makes it useless for bio-diesel production. Along with this other technologies e.g., biogas can mainly be used for electricity or thermal energy and not as a vehicle fuel. The designing and operation of a biomass gasification plant involves a number of factors and most of these are critical that may increase the chances of malfunctioning of the plant [5]. Another main energy conversion technology is biodiesel; it is produced from vegetable oils and animal fats through the process of trans-esterification. Mostly, it is obtained from *Pongamia, Jatropha* and other crops such as mustard, jojoba, flax, sunflower, palm oil, coconut etc. Several researchers have used crops such as rubber seed [7], jatropha [8,9], mahua [10], tobacco seed [11], castor [12], *Eruca sativa* [13] and pongamia [14]. Major lignocellulosic biomass does not contain oils which are essential for the production of biodiesel and some crops such as mustard which contain oils are consumed as animal feed. Moreover, the viscosity of neat vegetable oil (range of 28–40 mm^2/s) is high due to which its direct use has led to diesel engine problems such as deposits formation and injector coking arising from poor atomization [15]. In India, the recent studies have reported that the present economics of molasses-based ethanol production is not in the favor of commercial blending of ethanol in petrol. The study has indicated that if the government is targeting to bring into effect 10% blending by the year 2016–2017, as planned in the National Biofuel Policy, production of approximately 736.5 million t of sugarcane with area coverage of 10.5 million ha would be required [16] which is not feasible solution and will require huge investment. Moreover, it would be highly unsustainable to extend the sugarcane area beyond a certain limit, as the sugarcane is a highly water-intensive crop with water requirement of 20,000–30,000 m^3/ha/crop [16]. Therefore, it is necessary to find out an alternative way for the production of ethanol. Lignocellulosic substances such as cereal straws are available in large quantities and can be easily fermented to produce ethanol, which can be used either as a motor fuel in pure form or as a blending component in gasoline. Otherwise, farmers burnt these straws openly for clearing the field that led to the air pollution and emission of greenhouse gases. [17].

There are so many lignocellulosics agricultural waste available for ethanol production such as sugarcane baggase, rice hull, timber species, willow, salix, switch grass, softwood, rice straw, wheat straw etc. (Fig. 1.).

In agricultural dominating countries like India the crop residue and waste have great potential for ethanol production. Table 1 shows total crop residue production and their availability for ethanol production in India.

One can see there is significant increase in the availability of crop residue for ethanol production; it may be due to increased

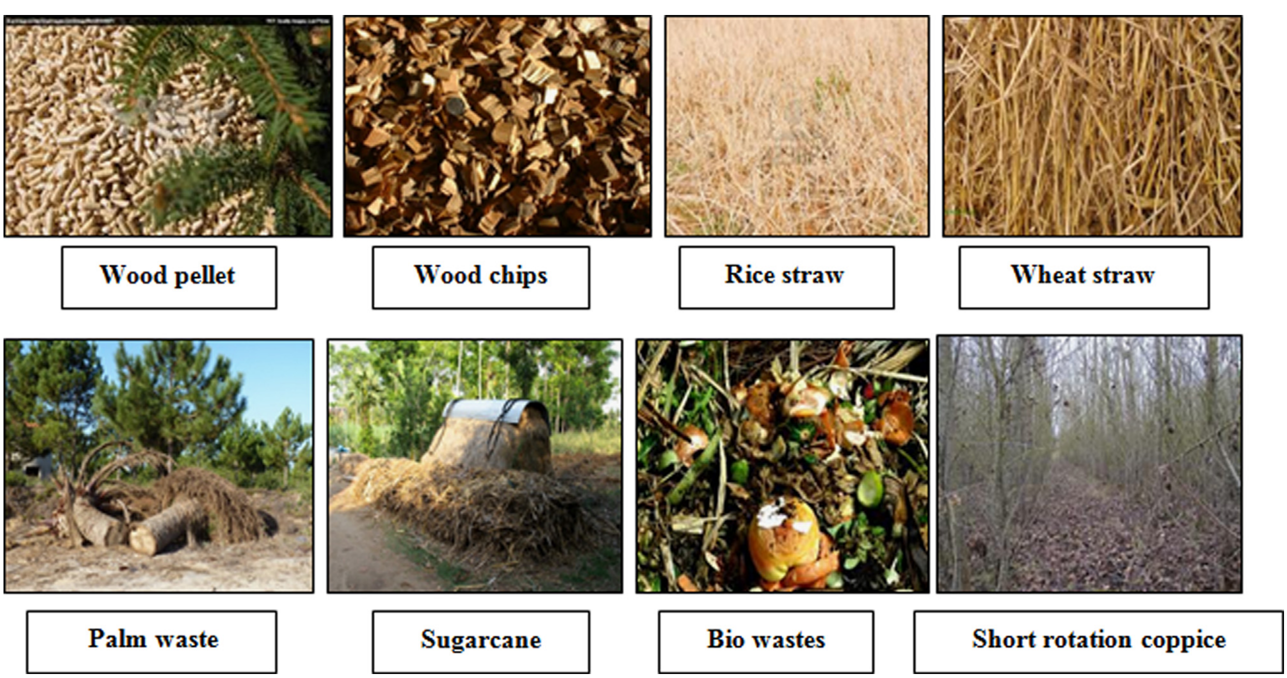


Fig. 1. Various lignocellulosic agricultural waste and crop for ethanol production.
Source: Adapted from [18].

Table 1
Crop residue production and their availability for ethanol production in India (Mt/year) [19].

State	Crop residues generation [20]	Crop residues surplus [20]	Crop residues burnt [21]	Crop residues burnt [22]
Andhra Pradesh	43.89	6.96	6.46	2.73
Arunchal Pradesh	0.40	0.07	0.06	0.04
Assam	11.43	2.34	1.42	0.73
Bihar	25.29	5.08	3.77	3.19
Chhattisgarh	11.25	2.12	1.84	0.83
Goa	0.57	0.14	0.08	0.04
Gujarat	28.73	8.9	9.64	3.81
Haryana	27.83	11.22	6.06	9.06
Himachal Pradesh	2.85	1.03	0.20	0.41
Jammu and Kashmir	1.59	0.28	0.35	0.89
Jharkhand	3.61	0.89	1.11	1.10
Karnataka	33.94	8.98	3.05	5.66
Kerala	9.74	5.07	0.40	0.22
Madhya Pradesh	33.18	10.22	3.74	1.91
Maharashtra	46.45	14.67	7.82	7.41
Manipur	0.90	0.11	0.14	0.07
Meghalaya	0.51	0.09	0.10	0.05
Mizoram	0.06	0.01	0.02	0.01
Nagaland	0.49	0.09	0.11	0.08
Odisha	20.07	3.68	2.61	1.34
Punjab	50.75	24.83	9.84	19.62
Rajasthan	29.32	8.52	3.84	1.78
Sikkim	0.15	0.02	0.01	0.01
Tamil Nadu	19.93	7.05	3.62	4.08
Tripura	0.04	0.02	0.22	0.11
Uttaranchal	2.86	0.63	0.58	0.78
Uttar Pradesh	59.97	13.53	13.34	21.92
West Bengal	35.93	4.29	10.82	4.96
India	501.76	140.84	91.25	92.81

Table 2
Ethanol production potential from major agro residues available in surplus [23].

Feedstock	Annual availability (MMT)	Theoretical yield[a] (l/dry ton) [8]	Max production potential (billion liters)	Max production potential assuming 50% potential (billion liters)
Rice straw	8.9	416	3.70	1.85
Wheat straw	9.1	432	3.93	1.97
Baggase	6.4	428	2.74	1.37
Corn stover	1.1	422	0.46	0.23
Total			10.84	5.42

[a] Theoretical ethanol yield calculated as per the conversion factors of US Department of Energy [24].

agricultural productivity and mechanization of agricultural industry. Different crops have different potential for ethanol yield. Table 2 shows ethanol yield for different crop residue. The cost of production of ethanol depends upon the procurement cost of raw material, processing, transportation from the site to the industry, ethanol production cost, market price and policies. Table 3 shows the procurement prices for major agro-residues in India.

In India, seasonal availability of agricultural crop residues depend on different types of crops (Table 4) e.g., maize stalk have availability period from August till the end of December whereas cotton stalk have availability in January and March. This type of information is very important to ensure the raw material availability throughout the year for the ethanol industry.

Lignocellulose consists primarily of plant cell wall materials; it is a complicated natural composite with three main biopolymers—cellulose, hemicellulose, and lignin [27,28]. Ash and other minor compounds are also present in the lignocellulosic biomass. It is a heterogeneous complex of carbohydrate polymers and lignin [29] and contains 55–75% carbohydrates by dry weight. Lignocellulosics do not contain simple sugars that can be readily converted to ethanol. Beside this, they contain polysaccharides such as cellulose and hemi-cellulose which need to be converted to the

Table 3
Procurement prices for major agro-residues in India [25].

Crop	Residue	Basic material cost (Rs/t)	Likely price (Rs/t)
Rice	Straw	600–1500	700
	Husk	1500–4000	1700
Wheat	Straw	2000–2700	2500
	Bagasse	1350–1500	1500
Sugarcane	Tops	Not sold often	–
	Stover	800–1500	1000
Maize/corn	Husk	Not sold often	–
Cotton	Stalk	500-800	600
Chilli	Stalk	Not sold often	–
Jowar/sorghum	Stover	3000–5000	4000
Ragi/finger millet	Stalk	Not sold often	–

monosaccharide. Cellulose and hemicellulose are closely associated with lignin thus prevent the access to the hydrolytic agents unless the lignin is modified or removed by chemical and/or biological methods [30]. Cellulose is a major structural component of cell walls and it provides mechanical strength and chemical stability to plants. Cellulose is the β-1,4-polyacetal of cellobiose (4-O-β-D-glucopyranosyl-D-glucose) and since the cellobiose consists

Table 4
Seasonal availability of agricultural crop residues in India [26].

Residue availability	Jan.	Feb.	Mar	Apr	Ma	Jun.	Jul.	Aug	Sep.	Oct.	Nov.	Dec.
Maize stalk								■	■	■	■	■
Maize cobs							■	■	■	■	■	■
Cotton stalks	■	■	■									
Mustard husk				■	■	■						
Jute and mesta sticks							■	■	■	■	■	■
Rice husk				■	■	■	■	■	■	■	■	■
Ground nut shells				■	■	■				■	■	■
Arhar stalks	■	■	■							■	■	■

Table 5
Composition of some lignocellulosic biomass (% of dry matter).

	Cellulose	Hemicellulose	Lignin[a]	Acid detergent lignin	Crude protein	Ash	References
Crop residues							
Corn stover	38	26	19	4	5	6	[39,46]
Soyabean	33	14	–	14	5	6	[40,41]
Wheat straw	38	29	15	9	4	6	[42,43,46]
Rye straw	31	25	–	3	3	6	[44,45]
Barley straw	42	28	–	7	7	11	[42]
Grasses							
Switch grass	37	29	19	6	3	6	[39,42,46]
Indian grass	39	29	–	6	3	8	[45]
Miscanthus	43	24	19		3	2	[47–49]
Reed canary grass	24	36		2	10	8	[40,50,51]
Other crops							
Sweet sorghum	23	14	11			5	[46]
Pearl millet	25	35		3	10	9	[40,50]

[a] Lignin is total lignin (acid soluble lignin+acid insoluble lignin).

of two molecules of glucose, it is considered as a polymer of glucose.
The specific structure of cellulose helps in the ordering of the polymer chains into a tightly packed, highly crystalline structure that is water insoluble and resistant to depolymerization [31]. Hemicellulose is the another carbohydrate component present in the lignocellulosic biomass that is composed of 5- and 6-carbon sugars and has a random, branched and amorphous structure with little strength. Both the cellulose and hemicellulose fractions are polymers of sugars and thereby a potential source of fermentable sugars that can be easily processed into different products [32–35]. Though there are many problems in producing ethanol from crop residues, presence of lignin is one of major constraint. Lignin is a highly stable biopolymer built from three cross-linked phenylpropane (C_6–C_3) units of p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol [36] which are bonded together with over two-thirds being ether bonds (C–O–C) and the rest C–C bonds [37]. Lignin can be hydrolyzed via cleavage of the ether bonds that are catalyzed by $[H^+]$ and $[OH^-]$ or water molecules [38]. Ash content is generally composed of minerals such as silicon, aluminum, calcium, magnesium and sodium. Other minor compounds present in the lignocellulosic biomass are extractives that include resins, fats and fatty acids, phenolics, phytosterols, salts and minerals. Composition of some lignocellulosic biomass is given in Table 5.

Lignocellulose bio-refineries via biological conversion generally have three main steps: (1) lignocellulose pretreatment, which converts the recalcitrant lignocelluloses structure to reactive cellulosic intermediates; (2) enzymatic cellulose hydrolysis, by which cellulases hydrolyze reactive intermediates to fermentable sugars (e.g., glucose and xylose); and (3) fermentation, which produces cellulosic ethanol or other bio-based chemicals (e.g., lactic acid, succinic acid) [52–55].

Fractionation of Lignocellulosic biomass is shown in Fig. 2. Fractionation contains two main parts recalcitrant and biodegradable. Opening up the recalcitrant structures would enable their biodegradability [56]. In which recalcitrant part requires pretreatments. There are many reasons [57,58] for using pretreatment and defined that an efficient method should have some major features summarized below;

- no production or very limited amount of production of sugar;
- production of very limited amount or no, for lignin;
- high digestibility of the cellulose;
- high recovery of all carbohydrates;
- liquid fraction must have high solid and liberated sugar concentration;
- need a very little demand of energy and
- should be economic and cost effective.

The process of pretreatment is one of very daunting steps in whole process of ethanol production. Flow diagram of enzymatic ethanol production process [59] is given in Fig. 3. Authors [59]

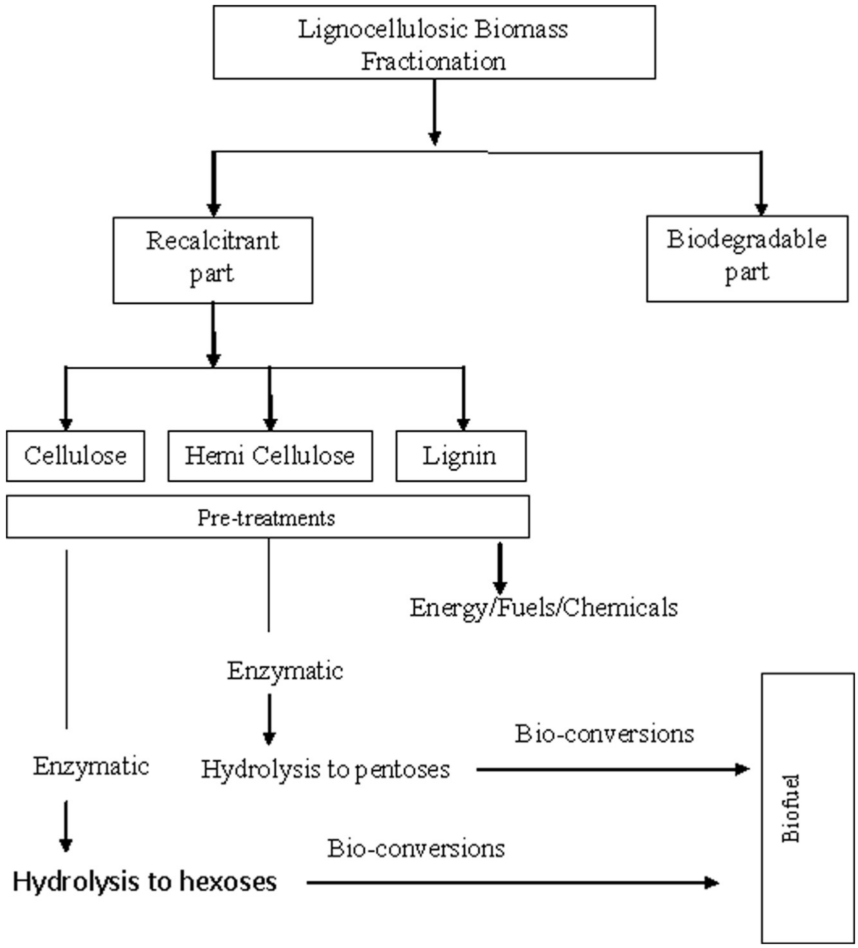


Fig. 2. Fractionation of lignocellulosic biomass.

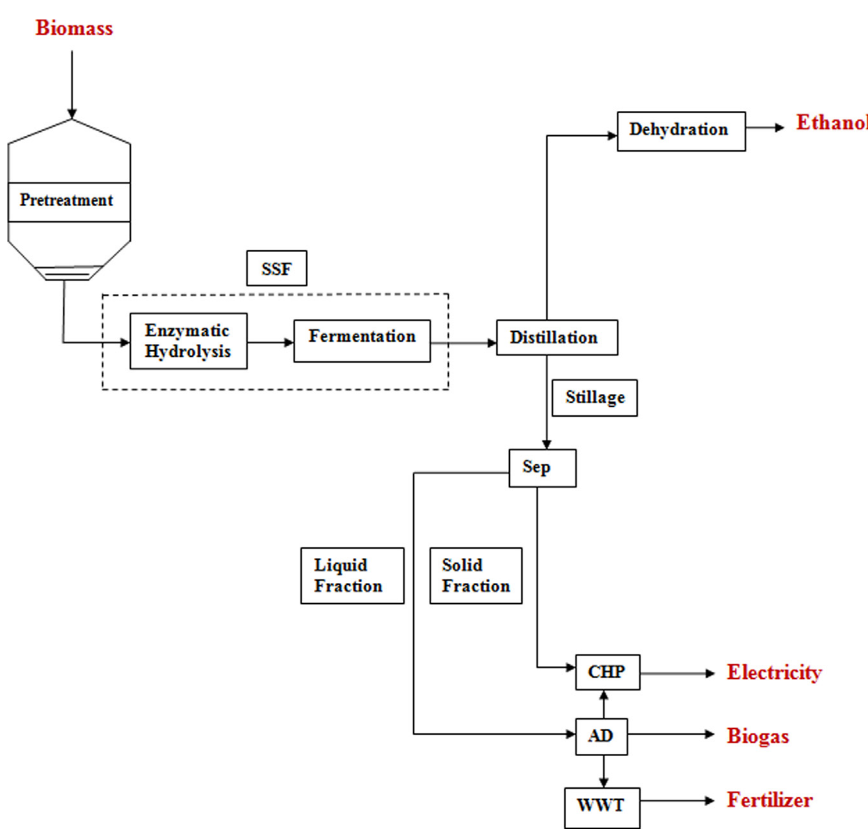


Fig. 3. Flow diagram of enzymatic ethanol production process [59].

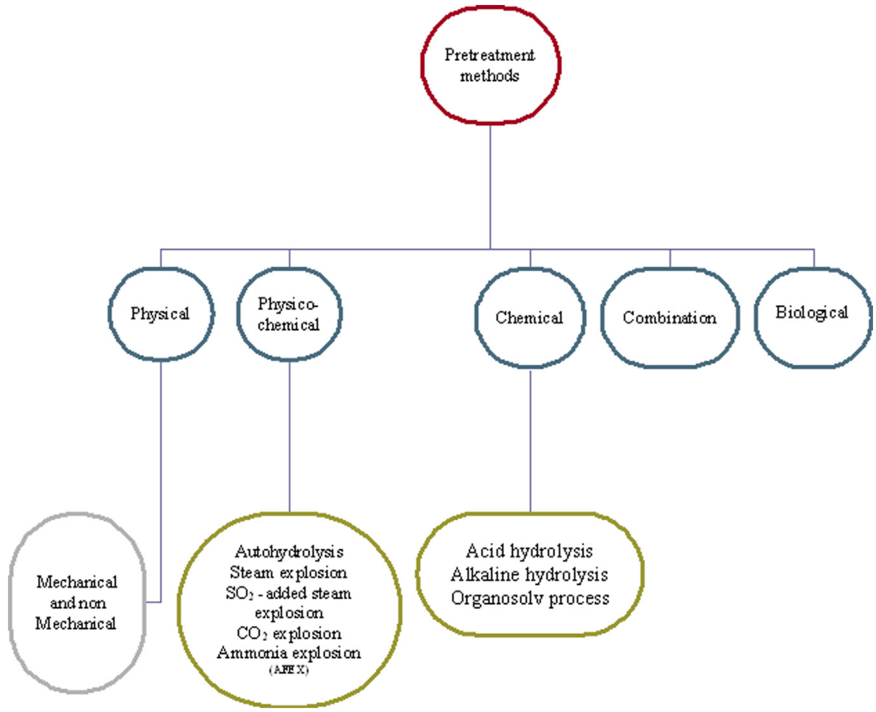


Fig. 4. Different pretreatment methods for delignification.

mentioned that pretreatment large impact on all other steps in the process.

2. Pretreatment of ligno-cellulosic substrates

The goals of the pretreatment are to decompose the polymeric components of the ligno cellulosic and form monomer sugars thus enhance enzymatic conversion of the cellulose fraction that increase the digestibility of the material for microbial and enzymatic bioconversion [60] and obtain a higher ethanol yield. Therefore, pretreatment is essential for the removal of lignin, reduction of cellulose crystallinity and increased porosity of the material. An effective pretreatment should produce significant percent of cellulose supports, the lesser production of inhibitors and be cost effective [61].

Various types of pretreatment methods have been investigated by the various investigators such as steam explosion, steam treatment with dilute sulfuric acid, or sulfur dioxide, organosolv pretreatment extraction, subcritical and supercritical water, and biological treatment with white rot fungi etc. [62].

Pretreatment methods can be categorized as physical, chemical or the combination of both and biological (Fig. 4). Physical pretreatment methods can further classified into mechanical and non-mechanical such as comminution and steam pretreatment respectively. Mechanical pretreatment reduces the biomass particle size that makes the lignocellulosic material more susceptible to chemical hydrolyzes. Non-mechanical physical treatment decomposes the substrate by exposing them to harsh external forces. When physical and thermo-mechanical processes combines, the energy requirement get increased that can make it uneconomical.

Chemical pretreatments are exclusively applied for the removal of lignin surrounding the cellulose and for destroying its crystalline structure. Conventionally, the paper and pulp industry has utilized delignification of wood to produce high strength, long fiber paper products. However these processes are quite expensive for the pretreatment of the lignocellulosics for the production of ethanol. Improvements in pretreatment, such as dilute acid hydrolysis, will aid in preparing feedstocks for enzymatic hydrolysis [63] and fermentation without generating significant concentrations of fermentation inhibitors [64]. After the steam pretreatment, the glucose released from cellulose is degraded to 5-hydroxymethylfufural (HMF), levulinic acids and formic acid whereas the pentose formed from hemicellulose is converted to furfural and formic acid. These degradation products, together with lignin degradation products release organic acids and act as inhibitors during fermentation and enzymatic hydrolysis [65]. Chemical pretreatments have serious disadvantages such as corrosion; need extensive washing and disposal of chemical waste [66]. Reaction rates for the generation of xylose from hemicellulose and the generation of furfural from xylose are strongly dependent on both temperature and acid concentration [64]. Phenol application promotes the decomposition of lignin into phenolic fragments by hydrolysis and pyrolysis. Phenol, along with the homogeneous reaction conditions also inhibits re-polymerization of the phenolics and promotes oil formation. The absence of phenol leads to the heterogeneity of lignin with water and inhibits the char formation [67].

3. Pretreatment methods

Pretreatment is one of the most expensive steps in biological conversion of cellulosic biomass. Pretreatment methods may be chemical, physical or hybrid in nature and mostly energy intensive. Some of the important pretreatment methods will be discussed in this section followed by appraisal of pretreatment methods.

3.1. Physical pretreatment

3.1.1. Mechanical comminution

Different types of mechanical size reduction methods such as coarse size reduction, chipping, shredding, grinding and milling are applied for enhancing the digestibility of lignocellulosic biomass [68]. These pretreatment techniques increase the specific surface area as well as decrease the degree of polymerization and cellulose crystallinity [61]. After chipping, the size of the materials

is about 10–30 mm and after milling or grinding, it is 0.2–2 mm. Both biomass characteristics and the final particle size required are the major factor on which energy needed for mechanical comminution depends. More energy is required for hardwoods than agricultural residues [69]. Different studies have reported that milling can increase bioethanol yields [70], but due to its high energy requirement on industrial scale, it is not economically sustainable [71]. Several studies have also evaluated that milling after chemical pretreatment will reduce (i) milling energy consumption, (ii) reduce cost of solid liquid separation because the pretreated chips can be easily separated, (iii) eliminate energy intensive mixing of pretreatment slurries, (iv) liquid to solid ratio and (v) did not result in the production of fermentation inhibitors [72,73]. Different types of milling processes such as vibratory milling, ball milling, disk milling, hammer milling are applied for enhancing enzymatic hydrolysis [74].Vibratory ball milling is more effective than ordinary ball milling in reducing cellulose crystallinity of spruce and aspen chips. Disk milling which produces fibers is more effectively enhances cellulose hydrolysis as compare to hammer milling which produces finer bundles [73].

3.1.2. Pyrolysis

Pyrolysis is another pretreatment method used for the lignocellulosic biomass. At higher temperatures, more than 300 °C, cellulose is decomposed into gaseous products and residual char. At lower temperature, the rate of decomposition is very slow. Mild acid hydrolysis (1 N H_2SO_4, 97 °C, 2.5 h) of the products obtained from the pyrolysis pretreatment can convert 80–85% of cellulose into reducing sugars with more than 50% is glucose [75].

3.1.3. Steam pretreatment

In this process, biomass is treated with high pressure saturated steam at temperatures about 160–240 °C and pressures between 0.7 and 4.8 MPa. Different studies have reported that steam pretreatment can efficiently hydrolyze the hemicelluloses (partially), modify the lignin, increases the surface area and decreases the cellulose crystallinity and degree of polymerization [76]. Steam pretreatment of corn stover at 190 °C for 5 min using SO_2 as acid catalyst has been shown to give high sugar yields (almost 90% overall glucose yield and almost 80% overall xylose yield) after 72 h enzymatic hydrolysis [77].

After steam pretreatment, inhibition of hydrolysis is largely due to non-specific hydrophobic binding of lignin to the cellulose binding domain of the enzymes [78]. The extended delignification, with increasing temperature, strongly affects the strength properties [79]. Ethanol can be produced from lignocellulosic biomass using steam pretreatment followed by enzymatic hydrolysis and fermentation. The sugar yields, from both hemicellulose and cellulose are critical parameters for an economically-feasible ethanol production process [80].

3.1.4. Steam explosion

Steam rapidly heats the biomass to the target temperature without excessive dilution of the resulting sugars [81]. In steam explosion, biomass is treated with hot steam (180–240 °C) under pressure (1–3.5 MPa) followed by an explosive decompression of the biomass that destroy the rigid biomass structure. The sudden release of pressure helps in defibrillating the cellulose bundles that enhances the accessibility of the cellulose for enzymatic hydrolysis and fermentation [82]. The two stages of steam explosion are auto-hydrolysis and de-pressurization. During auto-hydrolysis stage, high temperature promotes the formation of acetic acid from acetyl groups connected with hemicellulose, which leads to hemicellulose hydrolysis. The acetic acid formed further catalyzes the hydrolysis of the hemicelluloses. Depressurization stage reduces the biomass particle size and opens up particulate structure of biomass that enhances the enzymatic accessibility of cellulose. Steam explosion is one of the efficient pretreatments for corn stover biomass [83]. This process results in to the remarkable breakdown of the lignocellulosic structure, hydrolysis of hemicellulosic fraction, depolymerization of the lignin components and defibration. This process increases the accessibility of enzymes [84]. The main advantages of steam explosion pretreatment process are less use of hazardous chemicals, high energy efficiency and low environment impact [85]. Generation of toxic chemicals during the process and incomplete disruption of lignin are the two major shortcomings of this process. Steam explosion seems the best suitable physical pretreatment of straw as it partially hydrolyzes hemicellulose and increase the enzymatic digestibility of cellulose remaining in biomass residues [86].

3.2. Chemical pretreatment

3.2.1. Acidic pretreatment

For acid pretreatment, different studies has been done using sulfuric acid [87], hydrochloric acid [88], phosphoric acid [89] and nitric acid [90]. Several scientists have reported that the acid pretreatment enhances the hydrolysis of hemicelluloses and portion of amorphous cellulose and thus, increases the recovery of hemicelluloses as monomers in liquid fraction and digestible cellulose in solid fraction [91–93]. Although, the concentrated acids such as sulfuric acid and hydrochloric acid are powerful agent, they are not generally preferred because the concentrated acids are toxic, corrosive and hazardous in nature [94]. Dilute acid pretreatment (0.5–1.5%, temperature above 160 °C) appears more favorable method for industrial applications as high sugar yield is obtained from hemicelluloses, at least xylose yields of 75–90%. It can be performed at high temperature (e.g., 180 °C) for a shorter period of time or at low temperature (e.g., 120 °C) for longer period of time i.e., 30–90 min. It not only solubilises the hemicelluloses, but also converts it into fermentable sugars. Author [95] concluded that high saccharification yield (74%) is obtained when wheat straw was pretreated with 0.75% v/v of H_2SO_4 at 121 °C for 1 h. High ethanol yield (0.47 g/g glucose) was achieved when cashew apple bagasse was pretreated with diluted H_2SO_4 at 121 °C for 15 min [96].

3.2.2. Alkali pretreatment

Sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide ($Ca(OH)_2$) are generally used for alkali pretreatment. Alkaline hydrolysis involves the soapnification of intermolecular ester bonds cross linking xylan hemicelluloses and other components and the porosity of the lignocellulosic biomass increases with the removal of the cross links [97]. The efficiency of alkaline hydrolysis depends on the substrate and treatment conditions like it is more effective for low lignin content biomass such as agricultural residues. Sodium hydroxide increases the internal surface of cellulose, decreases the degree of polymerization and crystallinity and disrupts the lignin structure [74]. Author [98] reported that oxidative lime pretreatment of poplar at 150 °C for 6 h efficiently removed 77.5% of the lignin from the wood chips and also enhances the glucose yield. Oxidative lime pretreatment lowers the acetyl and lignin contents to obtain high digestibility, regardless of crystallinity [99]. A study has reported that for the pretreatment of rice straw using alkali chemicals i.e., NaOH, ($Ca(OH)_2$) and KOH in 24 h at 25 °C, among all the alkali chemicals, NaOH (6% chemical loading, g/g dry rice straw) was the best chemical that can achieve 85% increase of glucose yield by enzymatic hydrolysis [100]. Alkali removes acetyl groups from hemicellulose there by reducing the steric hindrance of hydrolytic

Table 6
Advantages and drawbacks of microorganisms used for bioethanol production.

Species	Characteristics	Advantages	Drawbacks	References
Saccharomyces cerevisiae	Facultative anaerobic yeast	Naturally adapted to ethanol fermentation High alcohol yield (90%) High tolerance to ethanol (up to 10% v/v) Amenability to genetic modifications	Not able to ferment xylose and arabinose sugars Not able to survive high temperature of enzyme hydrolysis	[109,110]
Candida shehatae	Micro-aerophilic yeast	Ferment xylose	Low tolerance to ethanol Low yield of ethanol Require micro-aerophilic conditions Does not ferment xylose at low pH	[109,111]
Zymomonas mobilis	Ethanologenic Gram-negative bacteria	Ethanol yield surpasses *S. cerevisiae* (97% of theoretical) High ethanol tolerance (upto 14% v/v) High ethanol productivity (five-fold more than *S. cerevisiae* volumetric productivity) Amenability to genetic modifications Does not require additional oxygen	Not able to ferment xylose Low tolerance to inhibitors Neutral pH range	[109,112]
Pichia stiplis	Facultative anaerobic yeast	Best performance xylose fermentation Ethanol yield (82%) Able to ferment most of cellulosic- material sugars including glucose, galactose and cellobiose Possess cellulose enzymes favorable to SSF process	Intolerant to high concentration of ethanol above 40 g/l Does not ferment xylose at low pH. Sensitive to chemical inhibitors Requires micro-aerophilic conditions to reach peak performance Re-assimilates formed ethanol	[109,113]
Pachysolen tannophilus	Aerobic fungus	Ferment xylose	Low yield of ethanol Require micro-aerophilic conditions Does not ferment xylose at low pH	[114]
Esherichia coli	Mesophilic Gram-negative bacteria	Ability to use both pentose and hexose sugars Amenability to genetic modifications	Repression catabolism interfere to co-fermentation Limited ethanol tolerance Narrow pH and temperature growth range Production of organic acid Genetic stability not proven yet Low tolerance to inhibitors and ethanol	[114,115]
Kluveromyces narxianus	Thermophilic yeast	Able to grow at high temperature above 52 °C Suitable for SSF/CBP process Reduces cooling cost Reduces contamination Ferment abroad spectrum of sugars Amenability to genetic modifications	Excess of sugars affect its alcohol yield Low ethanol tolerance Fermentation of xylose is poor and leads mainly to the formation of xylitol	[116,117]
Thermophilic bacteria ***Thermoanaerobacterium saccharolyticum*** ***Thermoanaerobacter ethanolicus*** ***Clostridium thermocellum***	Extreme anaerobic bacteria	Resistance to an extremely high temperature of 70 °C Suitable for SSCombF/CBP processing Ferment a variety of sugars Display cellulolytic activity Amenability to genetic modifications	Low ethanol tolerance	[117,118]

enzymes and greatly enhancing carbohydrate digestibility [101]. The release of lignin from the cell wall is mainly related to the hydrolysis. Alkaline pretreatment can be clubbed with the steam explosion to enhance the ethanol production [102].

3.2.3. Sulfur dioxide

Sulfur dioxide has a number of specific advantages as hydrolysis catalyst over H_2SO_4. Since SO_2 is a gas, it can be added either ahead of or with the steam; it is better distributed through the biomass than H_2SO_4, resulting in a more uniform reaction. Less SO_2 is used than H_2SO_4, and it costs less. Results of hydrolysis of several types of cellulosic biomass, namely pine sawmill residues, aspen poplar chips, sugarcane bagasse, wheat straw and corn cobs, has shown that hemicellulose was first hydrolyzed at 150 °C and residual cellulose was hydrolyzed at 190 °C, using SO_2 in both stages [103].

3.3. Biological pretreatment

Biological pretreatment for delignification from lignocellulosics is a safe and eco-friendly method for enhancement of susceptibility to enzymatic hydrolysis and digestibility [104–106]. The most promising class of white rot fungi is Basidiomycetes. Peroxidase produced by white-rot fungi weaken and/or break the bonds that bundle the cellulose and lignin together [107]. The extracellular enzymes from white-rot fungi include manganese peroxidase (Mnp; E.C.1.11.1.13), lignin peroxidase (Lip; E.C:1.11.1.14) and laccase (Lac; E.C:1.10.3.2). Among these enzymes Mnp plays the most important role for the degradation of lignin [108]. Table 6 shows the advantages and drawbacks of different microorganisms used in bioethanol process. Biological pretreatment is an eco-friendly process but the rate of biological pretreatment is very slow for industrial purposes. There are many drawbacks such as residence time of 10–14 days, careful growth conditions, large amount of space and high cost of enzymes which make this process economically less attractive on industrial scale. Also, some of the carbohydrate fraction is consumed by the microorganisms.

3.3.1. Enzymatic digestion

The optimization of enzymatic treatment, including the use of accessory enzyme such as xylanases and laccase can reduce the

concentrations of the enzymes needed and enhance the cost-effectiveness of ethanol production by enzymatic hydrolysis of lignocellulosics. The enzymatic digestibility of the lignocellulosics depends upon the extent of acetylation, lignification and crystallization [119]. The extensive delignification is sufficient to obtain digestibility regardless of acetyl content and crystallinity. Delignification and deacetylation remove parallel barriers to enzymatic hydrolysis and crystallinity significantly which affects initial hydrolysis rates but has less effect on ultimate sugar yields [119]. It has been reported that the particle size of biomass (excluding big chunks) has no effect on the enzymatic digestibility of corn stover [120], switch grass [119] and bagasse. Lee and Fan [121] reported that the rate of enzymatic hydrolysis depends on enzyme adsorption and the effectiveness of the absorbed enzymes. Lignin removal increases enzyme effectiveness by eliminating non-reproductive adsorption site by increasing access to holocellulose (cellulose and hemicellulose). The covalent linkages and physical binding between lignin and hemicellulosic grass cell walls [122] affects enzymatic hydrolysis. That is why enzymatic hydrolysis of wheat straw for ethanol production is bottle-necked by the cost of enzymes and the limitation of their efficacy [5].

3.4. Physico-chemical pretreatment

3.4.1. Wet oxidation (WO)

Wet oxidation (WO) is the process of treating material with water and air or oxygen at temperatures above 120 °C [123]. Combination of alkali and WO reduces the formation of toxic furaldehydes [124] and phenol aldehydes [125]. The wet oxidation increases cellulose content of baggase as a result of solubilization of hemicellulose and lignin [126].

A critical problem in the fermentation of dilute acid hydrolyzates is the inability of the fermentative microorganism to withstand inhibitory compounds formed during pretreatment, and usually a detoxification step is needed to improve hydrolyzate fermentability [127]. This was also true with the fermentation of the dilute acid hydrolyzates of wheat straw and rice hulls [128,129]. The inhibitor problem is not evident in the case of alkaline peroxide pretreatment of rice hulls. It is also being [124] reported that WO combined with base readily oxidizes lignin from wheat straw facilitating the polysaccharides for enzymatic hydrolysis. Furfural and HMF were not produced during the WO treatment. In the process water containing, dissolved hemicellulose and carboxylic acids was found to be a direct nutrient source for fungal growth and enzyme production. Unlike corn fiber hemicellulose, it is very resistant to hydrolysis using commercial enzymes. Rice hull hemicellulose can be easily hydrolyzed enzymatically by using a single xylanase preparation (Viscostar) after alkaline peroxide pretreatment [130].

3.4.2. Microwave irradiation

Microwave radiation by an electromagnetic field heats the object directly thus avoid temperature gradients. The deep penetration of microwaves into aqueous environments could make microwave heating appealing for pretreatment of cellulosic biomass by avoiding the temperature gradients typical for conductive methods [131,132] and overcoming the limitations on solids concentrations for mixed reactors [133]. The enzymatic hydrolysis

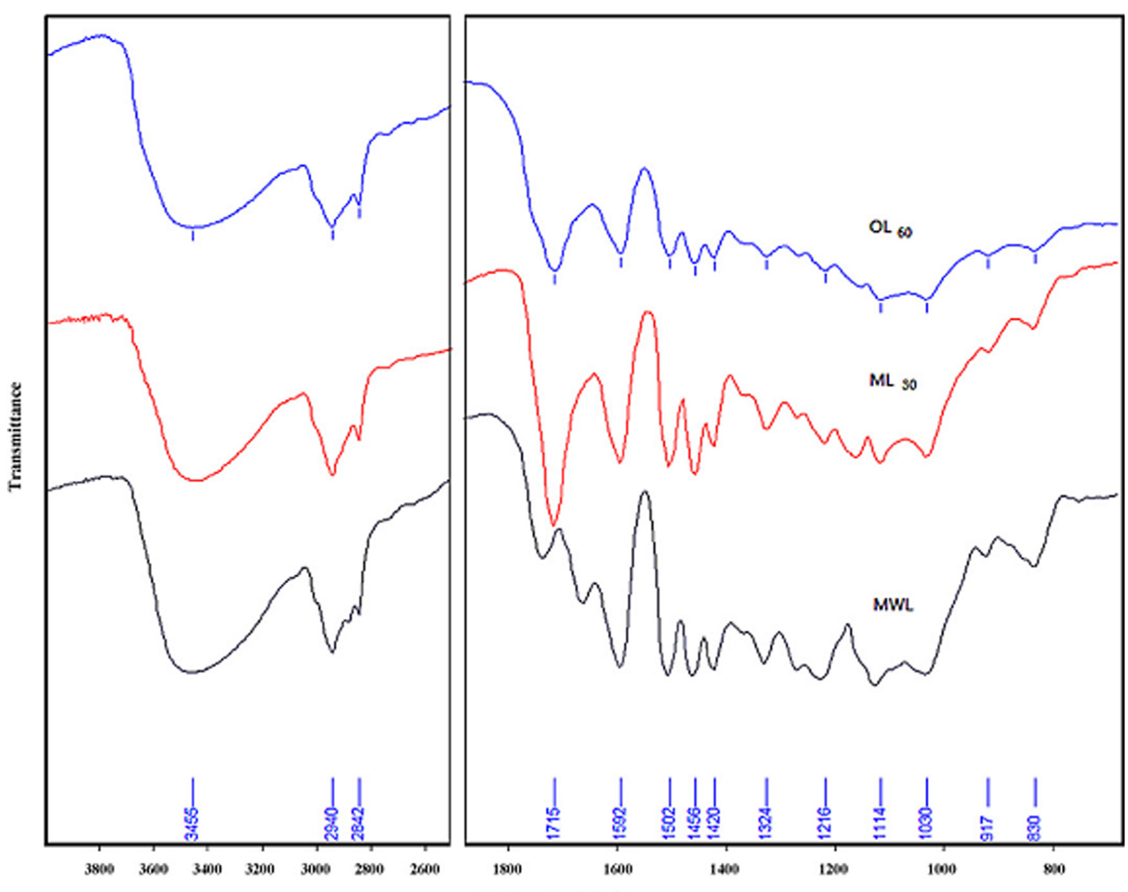
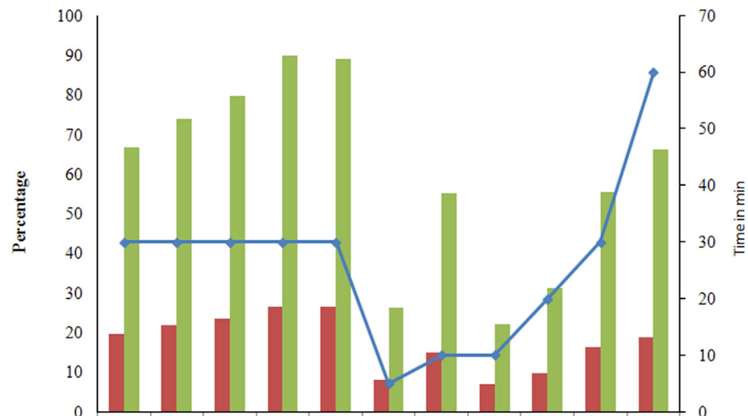


Fig. 5. FTIR spectra of birch formic acid lignins (ML30 and OL60) as compared to MWL [134].



	FL1 :Micro wave	FL2 :Micro wave	FL3 :Micro wave	FL4 :Micro wave	FL5: Micro wave	FL6 :Micro wave	FL7 : Micro wave	FL8 :Oil bath	FL9 :Oil bath	FL10 :Oil bath	FL11 :Oil bath
Extraction yield (%)	19.66	21.85	23.38	26.49	26.47	8.1	15.08	6.96	9.8	16.3	18.77
Delignification degree (%)	66.84	73.92	79.66	89.77	89.02	26.16	55.09	22.12	31.1	55.33	66.11
Heating time (min)	30	30	30	30	30	5	10	10	20	30	60

Fig. 6. Extraction yield and delignification degree under microwave heating and oil bath heating [134].

of biomass materials after microwave irradiation is an efficient pre-treatment technique. This process results in highest hydrolysis rate and glucose content in the hydrolysate [134].

Fig. 5 presents FTIR spectra of birch formic acid lignins (ML30 and OL60) as compared to MWL and stated that FTIR spectra of the lignin samples confirm feeble changes in the peak intensities. It does confirm that the "core" of the lignin structure did not change significantly during the fractionation processes [134]. It is also, concluded by authors that the lignin yield under microwave heating was much higher than that under oil bath heating.

It is quite clear from Fig. 6 that extraction yield and delignification degree both depends on heating time used in the process for microwave heating, and this effect is quite much dominating in microwave application with comparison to oil bath heating.

Microwave radiation employed for 10 min at 250 W to switch grass immersed in 3% sodium hydroxide solution (w/v) produced the highest yields of reducing sugar and thus combination of microwave-alkali pretreatment method has a potential to enhance enzymatic hydrolysis [135]. Microwave-chemical pretreatment is beneficial because of many reasons: (i) the microwave irradiation could enhance the lignin degradation and (ii) presence of aqueous NaOH increases soapnification of inter-molecular ester bonds cross-linking hemicellulose and lignin [136]. Several researchers have evaluated that microwave pretreatment in combination with other chemicals have been beneficial for pretreatment of rice straw [137,138]. Within loading range from 0 to 0.2 g alkali/g biomass, microwave removed more lignin as compare to conventional heating [139]. On the basis of temperature rise, moisture loss, heat capacity, heat of evaporation etc. the economics of microwave processing is evaluated [140]. The saponification of intermolecular ester bonds cross-linking xylan hemicelluloses and other hemicelluloses was enhanced by microwave irradiation [141]. Although, microwave heating enhances the cellulosic digestibility, specific surface area, hydrolysis of hemicelluloses and delignification, but it is highly energy intensive and therefore, excessively expensive. As a result, its implementation on commercial scale is difficult.

3.4.3. Ammonia fiber explosion

The AFEX pretreatment method is quite similar to the steam explosion method. In AFEX pretreatment, lignocellulosic biomass is exposed to liquid ammonia at a moderate temperature (60–100 °C) and a high pressure (250–300 psi) for a period of time and then the pressure is suddenly reduced. The operational parameters of AFEX are ammonia loading, temperature, water loading, blow down pressure, time and number of treatments [142]. Main advantages of this method are: high surface area, improved digestibility and enzyme accessibility (e.g., cellulase) [143], no toxic chemicals are generated for downstream processes. An important limitation of AFEX pretreatment method is that, it does not significantly remove hemicelluloses which may reduce enzyme accessibility and final sugar yield [144].

3.4.4. Liquid hot water

Liquid hot water (LHW) pretreatment uses water at elevated temperatures and high pressures to maintain its liquid form in order to promote disintegration and separation of the lignocellulosic matrix. Temperatures can range from 160 °C to 240 °C and over lengths of time ranging from a few minutes up to an hour with temperatures dominating the types of sugar formation and time dominating the amount of sugar formation. Xylose recovery is high (88–98%) and no acid or chemical catalyst is needed in this process, which makes it economically interesting and environmentally attractive [81]. However, it requires higher energy due to high pressures and a large amount of water.

4. Appraisal for pretreatment methods

It is being reported how pretreatment [59] has a very significant impact on other steps of ethanol production. Author [59] also mentioned formation of inhibitory substances during the pretreatment process. The variety of biomass also affects production of toxic compounds after pretreatment [59]. However in most of the pretreatment methods mainly furans, organic acids, aldehydes, ketones and aromatic compounds from lignin are toxic compounds which are generated. Pretreatment has large impact on all the other steps of the bioethanol process (Table 7). Furfural and HMF are the degradation products from pentose and hexoses which are the inhibitory substances formed during pretreatment. Effect of different pretreatment methods on sugar yield, formation of inhibitory substances, residue formation is shown in Table 8 [147]. It is therefore strong likelihood of having lower sugar and ethanol yield due to toxic compounds. Öhgren et al. [80] studied steam pre-treatment of corn stover with and without SO_2. Authors

Table 7
Characteristic features of some major ethanol production methods.

Method	Features	Reference
Enzymatic digestion	Particle size of biomass has no effect on enzymatic digestibility of certain crops; enzyme adsorption and the effectiveness of absorbed enzymes is major factor in enzymatic hydrolysis; Can reduce the concentration of enzymes needed; Extensive delignification is sufficient to obtain digestibility regardless of acetyl content and crystallinity	[119–121]
Wet oxidation (WO)	Treating material above 120 °C; Reduces the formation of toxics; Inability in fermentative microorganism in fermentation of dilute hydrolyzates	[123–126]
Steam explosion	Exposure to pressurized steam followed by rapid reduction in pressure; Lower environmental impact; Low chemical requirements; One of the most successful pretreatment method	[61,84,85]
Steam pretreatment	Combining withenzymatic hydrolysis and fermentation steam pretreatment provides good results	[80]
Sulfur dioxide	Works as hydrolysis catalyst; Due to gaseous nature can be ahead of or with steam; Provides more uniform and cost effective with pretreatment comparison to H_2SO_4	[103]
Acid and alkali pretreatment	Used for condensation reactions under acidic and alkaline conditions; Use of lime is widely accepted; Alkalis remove acetyl groups from hemicellulose and greatly enhancing carbohydrate digestibility; Combination with other pretreatment methods e.g., steam explosion, provides high yield	[101–103]
Biological pretreatment	Safe and eco-friendly; Biological pretreatment weaken and/or break the bonds that bundle the cellulose and lignin together	[104–106]
Microwave irradiation	The enzymatic hydrolysis of biomass materials after microwave irradiation is an efficient pre-treatment technique; Higher microwave power with short pretreatment time and the low microwave power with long pretreatment time had almost same affect; Added benefits of microwave pretreatment are: faster fractionation; high yield of lignin; lower yield of cellulosic pulp, no toxic substrate and more efficient	[62,151]

Table 8
Effect of some pretreatment methods on bioethanol process [147].

Pretreatments	Mode of action	Sugar yield	Inhibitor formation	Residue formation	Need for recycling chemicals	Additional remarks
Mechanical		−	++	++	++	
Weak acid	Removal of hemicelluloses (major) Alteration lignin structure (minor)	++	−	−	−	Specially suitable for biomass with low lignin content
Strong acid	Hydrolysis of cellulose and hemicellulose	++	−	−	−	Strong acid is hazardous, toxic and corrosive
Alkaline	Removal of lignin (major) and hemicellulose (minor)	++	++	−	−	
Wet oxidation	Removal of lignin (major) Dissolve hemicellulose Decrystallization cellulose	+/−	++	+	++	
Liquid hot water	Removal of hemicellulose	++	−	++	++	
Steam explosion	Removal of hemicelluloses (major) Alteration lignin structure (minor)	+	−	+	++	Low environmental impact
AFEX	Removal of lignin (major) and hemicelluloses (minor)	++	++		−	No need for small particle size for efficacy

+: positive characteristic (high yield of sugars, no or low inhibitors, no residue formation, no or low need for recycling of chemicals).
−: negative characteristics (low yield of sugars, high amount of inhibitors, high residue formation, need for recycling of chemicals).

[59,80] concluded that addition of small amount of xylanases had a very significant impact on xylose yield and reported that glucose yield increased from 69% to 94%. More or less same finding are also reported by other researchers [59,145,146].

5. Effect of delignification on physico-chemical structure of agri residues

Major physico-chemical changes that occur due to different types of pretreatments are: change in surface area, pore volume, biomass crystallinity, melting and relocation of lignin and degree of cellulose polymerization.

Crystallinity can affect the enzymatic saccharification of glucan. Different thermo chemical pretreatments can change cellulose crystalline structures by disrupting or destroying inter- or intra-hydrogen bonding of cellulose chains. Low pH pretreatments can enhance biomass crystallinity, while all high pH pretreatments have less effect on crystallinity. This shows that amorphous cellulose breaks down more at lower pH as compare to higher pH. Higher pH decrystallizes some of the cellulose [146].

Acetyl groups are side chains on the xylan backbone. It has been found that the removal of acetyl groups from biomass enhanced enzymatic digestion leads to enhance cellulose/xylan accessibility and enzyme effectiveness. Ammonia fiber expansion (AFEX) and lime pretreatments both removed the acetyl groups. But, AFEX remove the less while lime pretreatments remove the most acetyl groups. Although acetyl removal did not appear to be related to xylan removal for all pretreatments, it is apparent that higher xylan removal would result into greater acetyl removal [148].

The hydrothermal pretreatment process of delignification causes a partial defibrating effect on wheat straw; it produces a heterogeneous substrate of semi-separated fibers. It is found that the mostly, all fiber surfaces (more than 90%) are covered with

a layer of globular deposits. The deposits are established to re-localized lignin. After delignification of pretreated fibers, the cellulose fibrillar structure of the cell walls was found to be intact. The pretreatments had not disrupted the structure of the cell wall in order to increase its accessibility to enzymes. Partial hemicelluloses removal and lignin re-localization are important factors in increasing the digestibility of hydrothermally pretreated wheat straw. It is more important than rupture of the skeletal cell-wall structure and modification of cellulose crystallinity [149].

The surface morphology of the rice straw changed during its treatment with boiling hot acid solution. It is due to dissolution of characteristic species like releasing of silica, breaking of intra-molecular H-bonding of micro-fibril, and liberation of amorphous pectin, hemicellulose, and lignin. The basic plant components i.e., epidermis, vascular bundles and lumen were not largely changed its appearance during acid treatment. The alteration extent on cell wall after attacked by hot H^+ aqueous reaction was mainly depend on surface hydrophilic characteristics. There was less fractural damage on interior part of epidermis because of its wax-like hydrophobic properties, but there is a serious brash exfoliation on exterior area of epidermis due to hydrophilic nature of silica. There were little bulges and perforations showed on vascular bundles and tissue. Amorphous organic compound such as hemicellulose/cellulose, lignin and inorganic components like silica, trace metal element were easily removed by hot acid solution. An Acid treated straw had morphological and structural change on surface area and pore volume, as compare to untreated sample, both were greatly enhanced and further Hysteresis was widely decreased due to exposed more interior space that was supported by SEM inspection of some damages such as exfoliation, cave and fracture appeared on epidermis, vascular bundles. Crystallinity index of cellulose was increased but its crystalline was not changed because merely imperfect hemicellulose was removed while crystalline plane not changed [150].

The acid pretreatment of baggase samples shows that the amount of pith was reduced considerably, which indicates that pith is less resistant to acid degradation than the fiber structures that prevail. There were also some changed on fiber surfaces like: surface residual pith was removed and the parallel stripes appear more exposed [151].

Diluted alkali treatment of baggase samples shows the disruption of lignocellulosic cell walls by dissolving hemicellulose, lignin and silica, by hydrolyzing uronic and acetic acid esters and by swelling cellulose. Lignin decomposition is usually due to the cleavage of the α-aryl ether bonds from its polyphenolic monomers, while hemicellulose dissolution and cellulose swelling are due to the weakening of hydrogen bond. Sodium hydroxide (NaOH) presents the greatest degradation and subsequent fermentation yields when compared to other alkalis, such as sodium carbonate, ammonium hydroxide, calcium hydroxide and hydrogen peroxide. Lignin is the substance that gluing the sugarcane bundles together. Also, the gap between cellulose micro fibrils inside the walls is filled with a thin layer of the lignin hemicellulose complex. Lignin removal from baggase samples resulted in the separation of cell bundles, forming long cellular structures which are well connected in the longitudinal direction, but separated from neighboring bundles. Cell bundles that were tightly packed together before the base treatments, started to dismantle under NaOH concentrations lower than 0.5%. It is totally separated in some regions of the bagasse under higher NaOH concentrations. This resulted in a damaged and porous morphology, followed by a loss of mechanical resistance. It shows that lignin removal lead to the unstructuring of the sugarcane cell wall through a process that occurs in two levels. The first level refers to the loss of cohesion between neighboring cell walls, while the second one corresponds to degradation inside the wall itself, caused by peeling off and hole formation. All these morphological changes are significant in improving enzymatic hydrolysis efficiency, as enzymatic action is slowed down when the bagasse fibers are packed and their surfaces are protected [151].

The pretreatment of wheat straw with alkaline hydrogen peroxide released a maximum of about half of its lignin as water-soluble degradation products. Due to this treatment, the straw particles disintegrated into small, highly dispersed fibers, and the suspension acquired a more homogeneous, pulp like consistency. When the alkaline peroxide pretreatment is done with swirling provided by a gyro-rotary shaker (200 rpm) only a small amount of the straw disintegrated in 24 h. But if stirring was provided by a magnetic stirring bar, much more of the straw disintegrated in 24 h [152] all these changes help in increasing enzymatic digestion.

In corn stover, the hemicellulose fraction is in amorphous form. Lignin is closely associated with cellulose fibers and acts as a binder. They both are in the non-crystalline zone of the biomass. Their removal leads to increase in the surface area and porosity within the biomass; hence it provides easier access of enzyme to cellulose. Pretreatment of corn stover by aqueous ammonia is highly effective in enhancing enzymatic digestibility and reducing lignin content. The ammonia recycled percolation (ARP) process removed 70–85% of the total lignin and solubilized 40–60% of hemicellulose, but retained more than 95% of the cellulose. The surface area and crystalline index of ARP treated samples increases. Xylan and lignin both are present in amorphous form ARP treatment removed it. So, the Crystallinity Index increased after ARP treatment. However, there is no indication that the crystalline structure of the glucan content of the biomass is changed because of ARP treatment [153].

The morphology of wheat straw under various steam explosion process shows that the sizes of wheat straw particles become smaller at a higher steam temperature and longer retention time. The number of fibers also increases at this condition. The length of fiber becomes short. During the steam explosion process, the rapid thermal expansion helps in opening the particulate structure of the biomass. The rigid, ordered structure of biomass destroyed. The individual vascular cells separated due to disruption of middle lamella lignin as the cellulose becomes porous, loose. Due to porous structure, the enzyme could easily penetrate into cellulose and thus it enhances the yield of enzymatic hydrolysis [154].

Different groups of white-rot fungi, brown-rot fungi use different mechanisms in lignin degradation for different wood decay mechanisms. Brown-rot fungi include two groups. The first group could be *Gloeophyllum trabeum* (syn. *Lenzites trabea*)-type fungi, and the second group includes *Coniophora puteana* and *Poria* (Postia) placentatype fungi. Among brown-rot fungi, *G. trabeum* has been mostly studied. The initiators of both cellulose and lignin breakdown are suggested to be small molecular weight compounds that can readily diffuse from the hyphae and penetrate into the wood cell and start decay [155]. The morphological changes in cell walls after selective delignification provide important information regarding the mechanism of ligninolytic activity. Within the cell lumen, a lignin degrading system is produced. It consists of extracellular enzymes or possibly an oxidizing system that does not cause excessive degradation of the secondary wall but destroys the compound middle lamella. The absence of middle lamellae and loss of more than 95% of the lignin within the cell wall indicates the efficiency of these fungi for lignin removal. The extensive degradation of lignin also suggests that a highly diffusible ligninolytic system was produced during delignification that caused degradation at considerable distances from the fungal hyphae. In white-rotted wood, the lysis of the cell wall takes place around the hyphae that, results in a grove or trough. The formations of these erosion troughs in the cell wall suggest that enzyme diffusion is restricted to the immediate vicinity of the hyphae. Two different ligninolytic systems are produced by these white-rot fungi [156].

Table 9
Ethanol potential after different pre-treatment.

Substrate	Treatment	Ethanol recovery	References
Corn stalk	H_2SO_4	196 kg/t	[157]
Rice straw	Dilute H_2SO_4	6.5–11.35 g/l	[158]
Sugarcane leaf litter	H_2O_2	1.30 g/l	[159]
Sugarcane leaf litter	H_2SO_4	3.35 g/l	[159]
Waste cotton	H_2SO_4	14.2 g/l	[160]
Wheat straw	H_2SO_4	19 g/l	[128,129]
Corn cob	Enzymatic	95.3 g/l	[161]
Agave	HCl	7.4 g/l	[162]
Sugarcane bagasse	HCl	4.7 g/l	[162]
Agave	Alkaline-enzymatic	6.6 g/l	[162]
Sugarcane bagasse	Alkaline-enzymatic	12.9 g/l	[162]

6. Effect of pre-treatment on ethanol production

The ethanol production after the pretreatment depends on percentage of sugar recovery, type of simple sugar (pentose or hexose) and production of inhibitors. Table 9 shows the effect of some treatments on the ethanol recovery from the different substrates. It is quite evident that same types of pretreatment have significant difference on various types of crops e.g., using H_2SO_4 on sugarcane leaf litter (3.35 g/l) and Wheat straw (19 g/l). The ethanol recovery mainly depends on type of crops rather than pretreatment used. It also makes worth to notice that every crop need to have more optimum pretreatment method in order to have maximum ethanol recovery e.g., sugarcane leaf litter gives lower ethanol recovery if H_2O_2 is used in place of H_2SO_4 (Table 9).

7. Uses of lignin

However as discussed above lignin does affect the production of ethanol and need to be removed for better production efficiency but it had several uses reported in literature. Some of the major uses are discussed below [163,164]:

Lignin can be used as a low grade fuel by the direct combustion and it cannot be upgraded to oil, gas or recovered as chemicals [67]. Supercritical water can used a weak polar solvent to dissolve and hydrolyze lignin for potential production of phenolic chemicals or for upgraded fuel [67].

- Low levels of lignin and modified lignin can yield high performance concrete strength aid and concrete grinding aid. It can reduce damage of building external wall caused by moisture and acid rain. Sulfonated lignin contributes higher adsorption properties and zeta potential to cement particles, and hence shows better dispersion effect to the cement matrix.
- Lignin provides thermal protection to styrene/butadiene/rubber polymer, rubber, Polypropylene, polycaprolactam. Lignin's natural antioxidant properties provide use in cosmetic and topical formulations.
- Lignin amine additive has been shown to provide a warm mix additive that can modify the combination state of asphalt and stone material surface; modifying the fluidity; and decrease production cost of the asphalt mixtures. Water stability of an asphalt mixture can be improved by adding 0.3% lignin fibers.
- Native lignin or industrial lignin can be used for carbon fibers. Carbon nanotubes have been made from lignin/lignosulfonates. Lignin-based activated carbon fibers have been prepared by initial synthesis of lignin-phenol-formaldehyde (LPF) resins with varied lignin contents, 8–20%, respectively, and then the melt spinning and thermal treatments.
- Lignin along with a di-isocyanate used for production of fiberboards, strawboards, particleboards, oriented strand boards, wood fiber insulation boards, etc.
- Polyphenylene oxide-based polymers and lignin esters blends exhibit modulus of elasticity, tensile strength, and elongation at break values that are comparable or greater than the polyphenylene oxide-based polymer alone. The blends provide properties comparable to the polyphenylene oxide-based polymers, yet utilize fewer polymers.
- Lignin and glycerin in water applied upon the surfaces of dust-yielding situations like coal mines, coal transportation by rail car, and stock yards, etc. Dust movement can also be controlled by spraying a road surface with an emulsion of asphalt, lignosulfonic and water.
- Phenols prepared by taking lignin reacting with a H-supplying solvent at elevated temperature/pressure. Lignin depolymerization provides routes to Cresols, Catechols, Resorsinols, Quinones, Vanillin, and Guaiacols.
- Lignin enhances performance of energy storage devices. Lignin forms a thin layer on the graphite powder surface which prevents the graphite powder from decreasing H overvoltage and does not affect condition of the graphite powder.
- Indulin AT lignin when added to wood pellets produces better quality pellets and had higher fuel value.
- Grease with a wax/lignin has improved corrosion protection properties. Lignin sulfonate provides antifriction properties to grease providing longer lubrication life.
- A mixture including polycarboxylic acid and lignin sulfonic acid has been used for cleaning aluminum plates to prevent calcium scaling.
- Chemically modified lignin has been used as a dispersing agents, complexing agent, flocculent, thickener or auxiliary agents for coatings, paints or adhesives.
- Oxidized and pulverized lignin when blended with other chemicals can be used as a soil water retention agent in acidic dry land or desert soil, or as a binder for fertilizer.

8. Conclusions

The accessible surface area is the key determinant for the extent of delignification. Delignification with single method is not very effective for the further utilization of substrate for the ethanol production. The focus of the research should be on the development of combination of the delignification method on the basis of the lignin and cellulose profile of different potential raw material for the ethanol production. Energy auditing and the feasibility study should also be done along with the experiments. Some of the pretreatment methods have been found more suitable for certain types of crops due to their added advantage over others. However, lignin is material need to be removed by pretreatment methods in order to enhance ethanol yield, but there are several application being reported in recent years where lignin can be utilized in paint industry, agro farmland improvements, chemical coatings, cleaning metallic surfaces, dust particle controls and construction industry.

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